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SHORT COMMUNICATIONS

Cell Concentrations of Microorganisms in Glacial and Lake Ice of the Vostok Ice Core, East Antarctica

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Subglacial Antarctic lakes, in particular the largest of them, Lake Vostok (East Antarctica), are currently considered as unique analogues of extraterrestrial ice conditions probably existing on the poles or under the surface of Mars, on the satellites of Jupiter (Europe), or Saturn (Enceladus). The possibility of detection of microorganisms in such extreme natural habitats attracts particular attention with regard to the evolution of life as a whole and the capacity of microorganisms for wide-ranged adaptation in particular. Although the presence of microbial cells in Lake Vostok has already been reported by the results of studies of the lake ice, i.e., frozen lake water [1, 2], special caution is needed for interpretation of the findings due to the high probability of contamination of low-biomass samples by foreign microflora [3, 4].

The objective of the present study was assessment of cell concentrations of microorganisms in the lake ice of Lake Vostok buried under the 4-km East Antarctic ice sheet and isolated from the surface environment for at least 14 million years [5, 6]. This ice was compared with the glacial ice of atmospheric origin of the ice sheet. The final goal of this research was to study the structure of microbial communities in the water column and sediments of Lake Vostok under extreme living conditions characterized by the absence of light, high pressure (~400 bar), low temperature (close to the freezing point), extremely low content of dissolved organic matter, and the predicted huge excess of dissolved oxygen (up to 700–1300 mg/l [7]).

The lake ice obtained by deep-borehole ice coring at the Vostok station below the 3538-m horizon is formed of frozen lake water to the glacier foot [8] and at present provides the only possibility for search for life in subglacial lakes in Antarctica. At the same time, since this ice was not cored for biological studies, a big and yet unsolved problem is its decontamination, by which we mean the removal of not only microbial cells but also any nucleic acids (DNA) [3].

The age of Vostok lake ice is estimated as 20 thousand years at the most [6]. This ice is notable for the fact that it is formed of large monocrystals, up to 1 m in size, and consists of two layers [8]. The upper layer, type 1 ice, has fine, sometimes clearly visible inclusions of mica–clay minerals, often containing solid rock particles [9]. According to the commonly accepted hypothesis, it is formed above the shallow-water bay located in the western part of the lake along the flow line of glacier ice in the direction to the Vostok station. The lower type 2 ice does not contain mineral particles and is supposedly formed above the deep-water part of the lake.

The samples to study were the segments of the Vostok core ice and the surface snow collected in a big amount (up to 10 kg) with the maximum precaution against contamination in the surroundings of the Vostok station (table). Samples were decontaminated in the cold and clean (class 10000) rooms of the Laboratory of Glaciology and Geophysics of the Environment (LGGE) (Grenoble, France) using a class 100 laminar cabinet and ELGA ultrapure water (Purelab Maxima, Veolia water, United Kingdom) containing less than 2 μg per liter DOC (dissolved organic carbon) and filtered through a 5000 Da membrane. The melting of processed ice and snow and concentration of the biomaterial were carried out in a clean room in a laminar cabinet (LGGE). The biomass was concentrated by centrifugation using two types of filtering cartridges (Centriprep YM-3 and Centricon Plus-70, Millipore, with 3000 and 5000 Da membrane filters, respectively) retaining not only cells but also dissolved DNA, if presents. All columns, solutions, and instruments were certified as "free from foreign DNA". The quantity of microbial cells was assessed by the method of flow cytofluorimetry using the SYBR Green-I dye and a BD FACSAria device.

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Vostok ice core samples from boreholes 5G and 5G-1: cell concentration of microorganisms

Type of ice	Sample[1]	Cells per ml
Surface snow	VP08-1 (4.0–4.3)[2]	0.02
	VP08-2 (4.0–4.3)[2]	0
Glacier ice	122[3]	1.9
	2005	2.4
	2054	3–24[6]
	3489[4]	0
Lake ice, type 1	3561	4–9[6]
	3608	0–19[6]
Lake ice, type 2	3613	3 (2.4–4.4 SR)[7]
	3621	2 (2.4–3.1 SR)[7]
	3622	0.6
	3635	4.7
	3650	3.1
	3650[5]	4777
	3659	12

[1] The number corresponds to the depth in meters (m).

[2] MEGA-SNOW experiment (snow collected in big amount, up to 10 kg; age over 50 years, i.e. before the appearance of humans in this area).

[3] Dry bore-hde, BH7.

[4] Glacier basal layer with mineral inclusions of bed rocks.

[5] Unprocessed ice surface (the test for external general contamination).

[6] Extreme values of several measurements are given.

[7] The data of Scott Rogers' group on fluorescence microscopy for the same ice segment (Rogers S., personal communication). For the interval of depths 3501–3610 m, the cell concentrations (cells/ml) were determined as 2.3 ± 0.3–12.3 ± 9.6 [11].

For detection and counting of microbial cells, direct methods of fluorescence, laser confocal, and scanning electron microscopy were used, as well as flow cytofluorimetry. Microbial cells were not observed in thoroughly processed ice and snow samples by any of the microscopy methods. Only the flow fluorimetry method was able to count the cells in concentrated water samples in the range of 0–24 cells per ml of water for the glacier and lake ice of both types (see the table). The obtained result, which proved to be 2–3 orders of magnitude less as compared with the data published previously [1, 2], provides the evidence of an extremely low biomass in ice, both of atmospheric origin and that formed from the water of Lake Vostok, and emphasizes the importance of strict decontamination procedures of ice samples [3]. Besides, the data obtained for glacier ice demonstrate that the 3–4-km ancient ice sheet of Central East Antarctica is not so much the archive of the past microbiota as a barrier preventing the contact of potential lake biota with the surface for at least 14 million years.

Thus, the lake ice as a whole is exceptionally pure, contains almost no microorganisms (bacteria), and is characterized by a very low and nonuniformly distributed biomass. It is indirect evidence for the Lake Vostok water column (at least the surface layer) should also be poor in microbial content if any.

As a whole, the question about the existence of life in Lake Vostok is still open. Based on the findings, it seems that Lake Vostok may be a unique lifeless giant oxygen-saturated water system on our "bacterial" planet Earth and by this could provide a unique experimental area for development of the methods of searching for extraterrestrial life on icy planets and satellites.

Further work on the problem of existence of life in Lake Vostok will include the study of both new lake ice samples and (upon entering the lake) the water column (depth under the hole is about 680 m) and, above all, the sediments of the lake. It is very likely that, in contrast to ice and water, sediments holding the information about the distant past come up with diverse and unique microbial communities [10].

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